(19) Eur päisches Patentamt
European Patent Office
Offic europ´en des brevets

(11) Publication number: **0 199 992**
A1

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 86104149.9

(22) Date of filing: 26.03.86

(51) Int. Cl.⁴: A 61 K 37/02
A 61 K 47/00

(30) Priority: 28.03.85 JP 61926/85

(43) Date of publication of application:
05.11.86 Bulletin 86/45

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI NL SE

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(54) **Adsorption-resistant peptide composition and use of benzalkonium or benzethonium chloride in the preparation thereof.**

(57) An adsorption-resistant peptide composition contains benzalkonium chloride and/or benzethonium chloride to prevent the peptide from being adsorbed on the inner wall of a container or equipment.

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SPECIFICATION

TITLE OF THE INVENTION:

ADSORPTION-RESISTANT PEPTIDE COMPOSITION AND USE OF BENZALKONIUM OR BENZETHONIUM CHLORIDE IN THE PREPARATION THEREOF

BACKGROUND OF THE INVENTION

a) Field of the Invention:

This invention relates to an adsorption-resistant peptide composition, and more specifically to an adsorption-resistant peptide composition which contains benzalkonium chloride or benzethonium chloride as an essential component.

b) Description of the Prior Art:

In general, many of peptides have physiological effects as peptide hormones for example. Upon their administration as drugs, their doses are very small, i.e., on the order of several micrograms and these doses must strictly followed. In other words, there is a special requirement that the above substances, which have been formulated in very small amounts into preparations, must be administered precisely in their formulated amounts.

It has however been well-known that proteins and peptides, which are contained in an aqueous solution, tend to adhere on glass or its analogous material and

as a result, their contents are caused to decrease considerably compared with their original amounts in a preparation.

It has also been known that when insulin is injected as an illustrative peptide in an infusion container, a substantial portion of the insulin is instantaneously adsorbed on the glass surface of the container and the adsorption is liable to occur at a lower insulin concentration ["Pharmaceutics", 39, 107-111 (1979)]. Since doses of peptide hormones such as insulin are limited to very small levels by their nature, they are in a state liable to easy adsorption on glass containers. Moreover, the rates of their adsorption loss reach significant levels because their initial contents are low, leading to considerable decreases to their actually-administered amounts.

Even if a peptide is accurately incorporated in a prescribed amount while paying special attention to containers and other equipment to avoid its adsorption upon formulation of a dosable preparation, it will be adsorbed in a significant amount on the glass wall of a syringe container or the glass wall of an infusion container when the preparation is transferred in the syringe cylinder or is injected in the infusion container for its mixed injection along with the infusion fluid upon actual administration of the

peptide. As a result, the amount of the peptide will be decreased to a considerable extent.

Plastic syringe cylinders (made of polypropylene), infusion bottles (made of polypropylene or polyethylene), instillator tubes (made of polyvinyl chloride) and the like have found wide-spread commercial utility in recent years. Peptides are therefore more liable to adsorption. With the foregoing in view, the present inventor conducted a research with a view toward developing a method for preventing adsorption of insulin, secretin and other peptides upon their contact to the plastic walls of various containers and equipment. As a result, it was found that the above object can be achieved by adding one or more substances, which are selected from the group consisting of lecithin, ethylene oxide-propylene oxide copolymers, hydroxypropylcellulose, methylcellulose, polyoxyethylene-hardened castor oil, polyethylene glycol sorbitan oleate, methylcyclodextrin and sorbitan fatty acid esters, to a composition which contains insulin, secretin or other peptide. The above finding led to completion of inventions, on which patent applications were filed under Japanese Patent Application Nos. 89807/1982, 186089/1982 and 122421/1983.

SUMMARY OF THE INVENTION

Thereafter, the present inventor proceed with a further investigation on adsorption of various peptides upon their contact with container walls and prevention of their adsorption. As a result, it has been found that the above object can be achieved by adding benzalkonium chloride or benzethonium chloride to an aqueous peptide-containing solution or suspension, leading to completion of the present invention.

In one aspect of this invention, there is thus provided an adsorption-resistant peptide composition comprising benzalkonium chloride or benzethonium chloride as an essential component.

Benzalkonium chloride and benzethonium chloride are both effective at an extremely low concentration, for example, at 0.001% or higher in preventing adsorption of a peptide on the wall of a container. The concentration of the peptide is therefore maintained constant in the composition of this invention. The peptide can therefore be administered in the same amount as it is incorporated upon preparation of the composition.

The above and other objects, features and advantages of this invention will become apparent from the following detailed description of the invention and

the appended claims, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 through 10 are histograms showing the relations between recovery rates of various peptides, namely, substance-P (Fig. 1), neurotensin (Fig. 2), ACTH (Fig. 3), oxytocin (Fig. 4), bradykinin (Fig. 5), neoendorphin (Fig. 6), dynorphin (Fig. 7), angiotensin I (Fig. 8), secretin (Fig. 9) and insulin (Fig. 10) and the type and concentration of an additive added in accordance with this invention. In each of the drawings, the lefthand histogram shows results obtained by using glass bottles while the righthand histogram illustrates results obtained by using plastic bottles.

DETAILED DESCRIPTION OF THE INVENTION

The term "peptide" as used herein may for example include insulin, secretin, P-substance, angiotensin I, bradykinin, neoendorphin, neurotensin, calcitocin, oxytocin, glucagon, ACTH and dynorphin. The molecular weights of peptides having physiological effects fall generally within a range of from 200 to 6,000 in many instances. The molecular weights of the above-exemplified peptides also fall within a range of from 1,000 to 6,000. Since the present invention can

exhibit useful preventive effects against all peptides which tend to adsorb on container or equipment walls, the present invention shall not be limited to any particular molecular weight range.

The adsorption-preventive additive is benzalkonium chloride or benzethonium chloride in the present invention as will be demonstrated in Experiments and Examples, which will be described herein.

It was found by the present inventor that these substances can be specifically selected for the prevention of peptide adsorption and they can exhibit almost perfect adsorption-preventive effects at considerably low concentrations. When the peptide-containing composition takes the form of an aqueous solution or suspension, benzalkonium chloride or benzethonium chloride may desirably be contained in an amount of 0.001 or higher in the aqueous solution or suspension. Its concentration may desirably be at such a low level that it does not show any pharmacological effects upon its in-vivo administration. For these reasons, it is preferable to incorporate benzalkonium chloride or benzethonium chloride in such an amount that its concentration is 1% or lower in a resulting aqueous peptide solution or suspension.

The lower concentration limit of the additive in the present invention is not determined by the content of an associated peptide but is governed by the surface area of the inner wall of each container. If the maximum surface area of a container or equipment with which a peptide-containing composition of this invention is brought into contact is known, it is possible to determine, in accordance with the maximum surface area, the amount of the additive to be added to the peptide-containing composition of this invention. In practice, it is however not feasible to know such a surface area in advance. It is hence impossible to determine precisely the content of such an additive in the composition. As will be demonstrated in the below-described Experiments, it is however appropriate in achieving the objects of this invention to control the concentration of the additive within the above-described concentration range, namely, to an amount of 0.001% - 1% in an aqueous solution or suspension when the peptide-containing composition takes the form of the aqueous solution or suspension.

The composition of this invention may take several types of preparation forms. It is not absolutely necessary to provide the peptide and the additive of this invention in such a form that they are both contained in the same composition from the

beginning. The composition may hence take such a form that the peptide and additive are provided separately and are mixed with each other upon administration of the peptide. The following preparation forms may be mentioned as illustrative embodiments of the composition:

i) A composition in which a peptide and its associated additive of this invention are both contained in the same aqueous solution or suspension.

ii) A kit in which a peptide and its associated additive of this invention take the form of different aqueous solutions or aqueous suspensions. When the peptide is administered, they are mixed with each.

iii) A composition in which a peptide and its associated additive of this invention are both incorporated in the same solid matter or powder. When the peptide is administered, a solution which has been separately furnished is added to the composition to form a solution or suspension.

iv) A kit in which a peptide and its associated additive of this invention are provided as separate solid matters or powders. When the peptide is administered, they are converted into aqueous solutions or suspensions and are then mixed.

v) A kit in which a peptide is provided as a solid matter or powder while its associated additive of

this invention is furnished as an aqueous solution. When the peptide is administered, they are mixed together.

The above-mentioned various aqueous solutions, aqueous suspensions, and solid matters or powders may be readily prepared by usual methods. It is also free to practice in the present invention to add one or more suitable stabilizers, buffer agents and/or the like in such aqueous solutions or aqueous suspensions and to incorporate one or more buffer agents and suitable solidifying or powderizing excipients in such solid matters or powders. In general, benzalkonium chloride and benzethonium chloride are employed singly. They may however be used in combination.

Effects of this invention will hereinafter be described by the following Experiments.

Experiment 1:

(Sample)

Solutions containing benzalkonium chloride respectively at 0.1%, 0.01% and 0.001% in a 0.9% aqueous solution of NaCℓ and solutions containing benzethonium chloride at the same concentrations in the same aqueous NaCℓ solution were prepared as sample solutions.

Besides, the 0.9% aqueous solution of NaCℓ was also provided as a control.

(Method)

The various peptides described in the column entitled "Peptide" in Table 1 were separately dissolved in water to prepare their 50 μg/mℓ solutions, which were used as neat solutions. The sample solutions were separately poured in an amount of 1 mℓ per bottle in plastic bottles and glass bottles. The neat peptide solutions were separately added, in an amount of 50 μℓ per bottle, to the plastic and glass bottles. After vigorously shaking the bottles, 50 μℓ portions of the resultant mixture were separately taken in microsyringes. They were separately injected in the column of a high-speed liquid chromatography instrument. The amounts of unadsorbed peptides were measured by the high-speed liquid chromatography instrument to determine their recovery rates.

The high-speed liquid chromatography instrument was operated at a measurement wavelength of 200 nm with mobile phases given in Table 1.

Table 1

Peptide	Mobile phase
P-substance	0.1-M $NaClO_4$(pH 3.0)-CH_3CN = 65:35
Angiotensin I	0.1-M $NaClO_4$(pH 3.0)-CH_3CN = 67:33
Bradykinin	0.1-M $NaClO_4$(pH 3.0)-CH_3CN = 65:35
Neoendorphin	0.1-M $NaClO_4$(pH 3.0)-CH_3CN = 65:35
Neoendorphin	0.1-M $NaClO_4$(pH 3.0)-CH_3CN = 65:35
Neurotensin	0.3-M $NaClO_4$(pH 3.0)-CH_3CN = 65:35
Oxytocin	0.3-M $NaClO_4$(pH 3.0)-CH_3CN = 75:25
ACTH	0.2-M $NaClO_4$(pH 3.0)-CH_3CN = 60:40
Dynorphin	0.3-M $NaClO_4$(pH 3.0)-CH_3CN = 62:38
Insulin	1.4% $HClO_4$-CH_3CN = 65:35
Secretin	0.2% $HClO_4$-CH_3CN = 60:40

Results:

Results are shown in Figs. 1 through 10, which correspond to P-substance, neurotensin, ACTH, oxytocin, bradykinin, neoendorphin, dynorphin, angiotensin I, secretin and insulin respectively. In each drawing, the lefthand histogram shows results obtained by using glass bottles while the righthand histogram illustrates those obtained by using plastic bottles.

In each drawing, "Cont." corresponds to recovery rates obtained from the use of the 0.9% aqueous solution of NaCℓ only. On the other hand, "BL" and "BT" indicate recovery rates obtained respectively when benzalkonium chloride and benzethonium chloride were added separately to the 0.9% aqueous solution of NaCℓ.

"A", "B", "C" and "D" indicate recovery rates of the respective peptides when the contents of the associated additives were 0.1%, 0.01%, 0.001% and 0.0001% respectively.

From Figs. 1 through 10, it is clearly envisaged that the additives of this invention serve as adsorption-preventing agents. It is also appreciated that the additives of this invention can each exhibit its effect and can hence prevent peptides from being adsorbed on containers or equipment when incorporated at a concentration of 0.001% or higher.

The present invention will hereinafter be described specifically by the following Examples.

Example 1:

An aqueous solution having a total volume of 100 mℓ and containing calcitocin (16,000 units), glycerin (2 g), benzalkonium chloride (0.10 g) and sodium chloride (0.9 g) was aseptically prepared. It was filled 1 mℓ by 1 mℓ in ampules, followed by their sealing.

Example 2:

An aqueous solution having a total volume of 100 mℓ and containing calcitocin (16,000 units) and glycin (4.0 g) was aseptically prepared. It was filled 1 mℓ by 1 mℓ in vials. The aqueous solution was solidified by lyophilization, followed by their sealing.

A 0.01% aqueous solution of benzethonium chloride was also aseptically prepared. It was filled 1 mℓ by 1 mℓ in ampules, followed by their sealing to provide an ampule-filled solution for dissolution.

Example 3:

An aqueous solution having a total volume of 100 mℓ and containing oxytocin (200 units), benzalkonium chloride (0.10 g) and sodium chloride (0.90 g) was aseptically prepared, followed by its aseptic adjustment to pH 2.1 - 4.5 with hydrochloric acid. It

was then filled 1 mℓ by 1 mℓ in ampules, followed by their sealing.

Example 4:

An aqueous solution containing L-alanine (4 g) and benzalkonium chloride (0.01 g) in 0.03 M citrate/disodium phosphate buffer of pH 4.0 (100 mℓ) was aseptically prepared. It was filled 1 mℓ by 1 mℓ in vials, followed by their sealing. Ampules containing injection-grade distilled water (1 mℓ) were also provided as ampule-filled water for dissolution.

Example 5:

An aqueous solution having a total volume of 100 mℓ and containing ACTH (2,500 units) and benzethonium chloride (0.10 g) was aseptically prepared. It was filled 1 mℓ by 1 mℓ in silicone-coated ampules, followed by their sealing.

Example 6:

An aqueous solution having a total volume of 100 mℓ and containing ACTH (2,500 units), 0.05 g of benzalkonium (0.05 g) and benzethonium chloride (0.03 g) was aseptically prepared. It was filled 1 mℓ by 1 mℓ in ampules, following by their sealing.

Example 7:

A 0.1% aqueous solution of benzalkonium chloride was aseptically prepared. It was filled 2 mℓ by 2 mℓ in ampules, followed by their sealing to provide an ampule-filled solution for the prevention of peptide adsorption.

Example 8:

An aqueous solution containing benzalkonium chloride (0.05%) and benzethonium chloride (0.05%) was aseptically prepared. It was filled 2 mℓ by 2 mℓ in ampules, followed by their sealing to provide an ampule-filled solution for the prevention of peptide adsorption.

Having now fully described the invention, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

CLAIMS:

1. An adsorption-resistant peptide composition comprising benzalkonium chloride or benzethonium chloride as an essential component.

2. An adsorption-resistant peptide composition as claimed in Claim 1, wherein the peptide is P-substance, angiotensin I, bradykinin, neoendorphin, neurotensin, calcitocin, oxytocin, glucagon, ACTH, dynorphin, secretin or insulin.

3. An adsorption-resistant peptide composition as claimed in Claim 1 or 2, wherein the composition takes the form of an aqueous solution or suspension and benzalkonium chloride or benzethonium chloride is contained in an amount of 0.001 - 1% in the aqueous solution or suspension.

4. Use of benzalkonium and/or benzethonium chloride in the preparation of an adsorption-resistant peptide composition.

CLAIMS FOR AUSTRIA

1. Process for the preparation of an adsorption-resistant peptide composition characterized in that benzalkonium chloride and/or benzethonium chloride are added to the composition.

2. Process according to claim 1 in which said adsorption-resistant peptide composition is a pharmaceutically acceptable mixture of components.

3. Process according to claim 1 or 2 in which the peptide is P-substance, angiotensin I, bradykinin, neoendorphin, neurotensin, calcitocin, oxytocin, glucagon, ACTH, dynorphin, secretin or insulin.

4. Process according to any of claims 1 to 3 in which the composition is prepared in the form of an aqueous solution or suspension and benzalkonium chloride and/or benzethonium chloride are added in an amount such that their total or final concentration is 0.001 to 1% w/v of the aqueous solution or suspension.

5. Use of benzalkonium and/or benzethonium chloride in the preparation of an adsorption-resistant peptide composition.

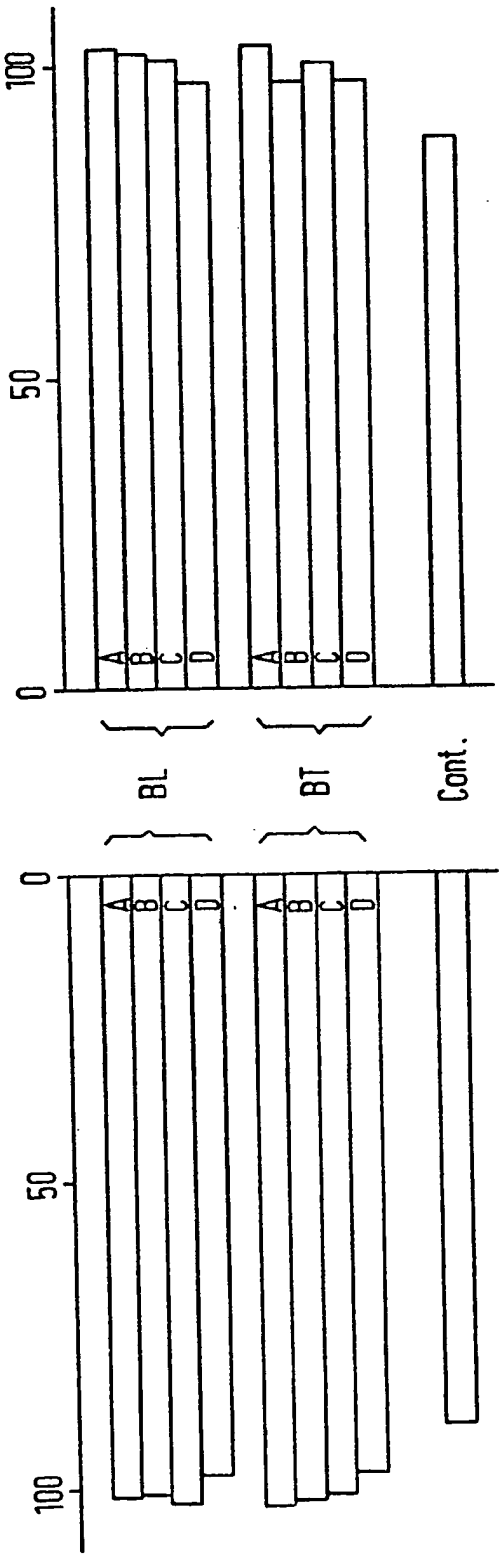

FIG. 1

FIG. 2

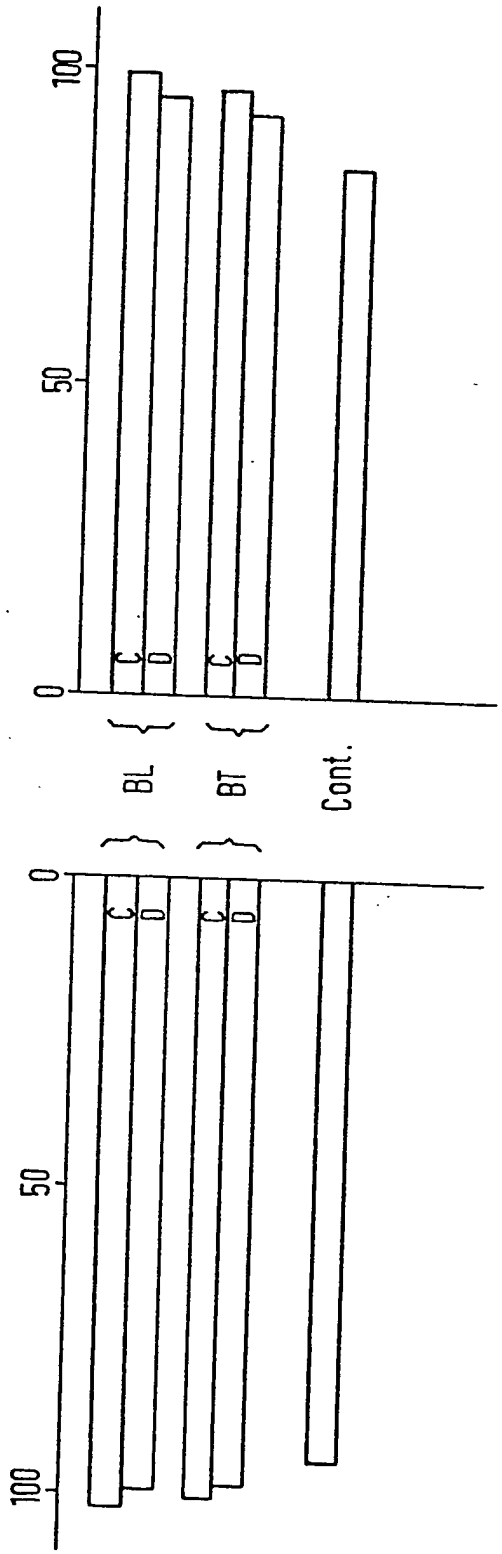

FIG. 3

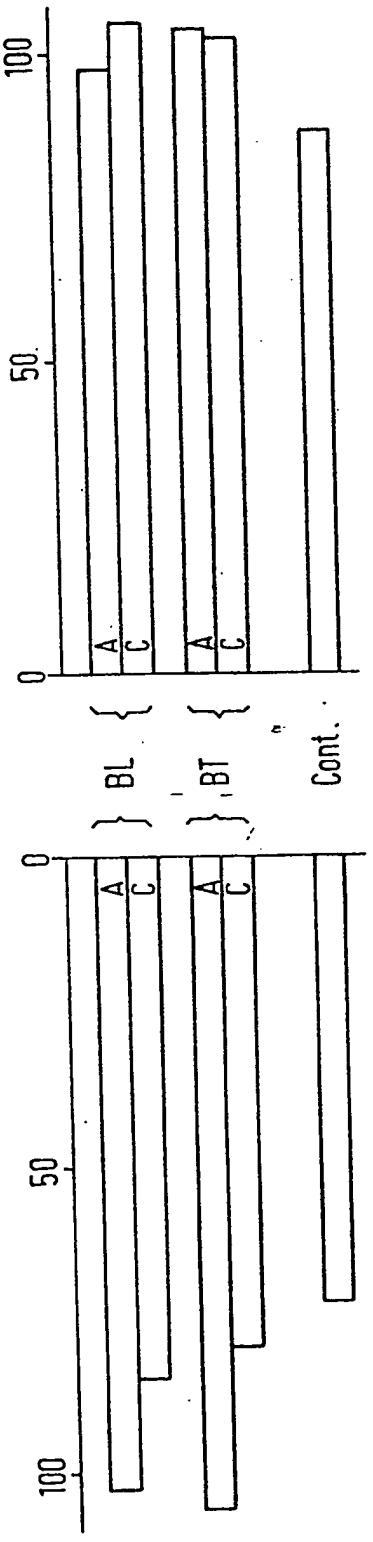

FIG. 4

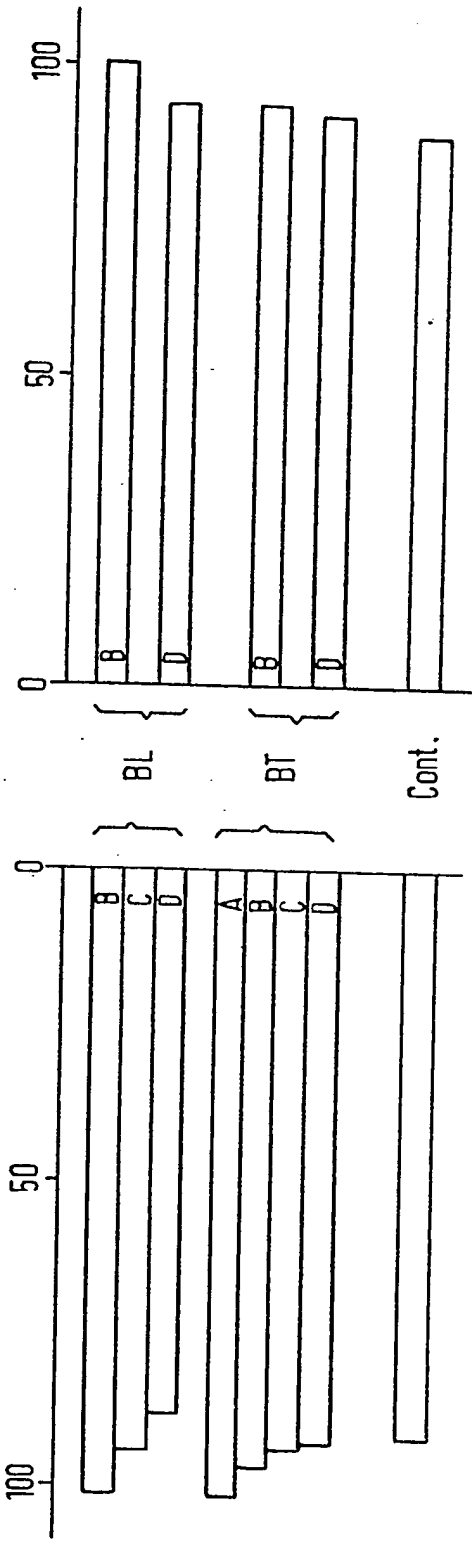

FIG.5

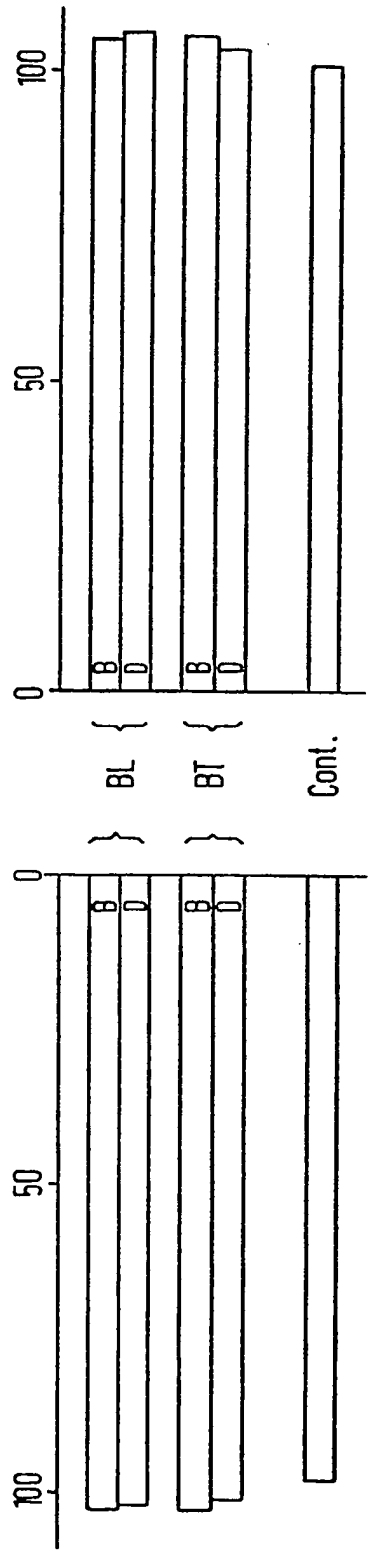

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FIG.6

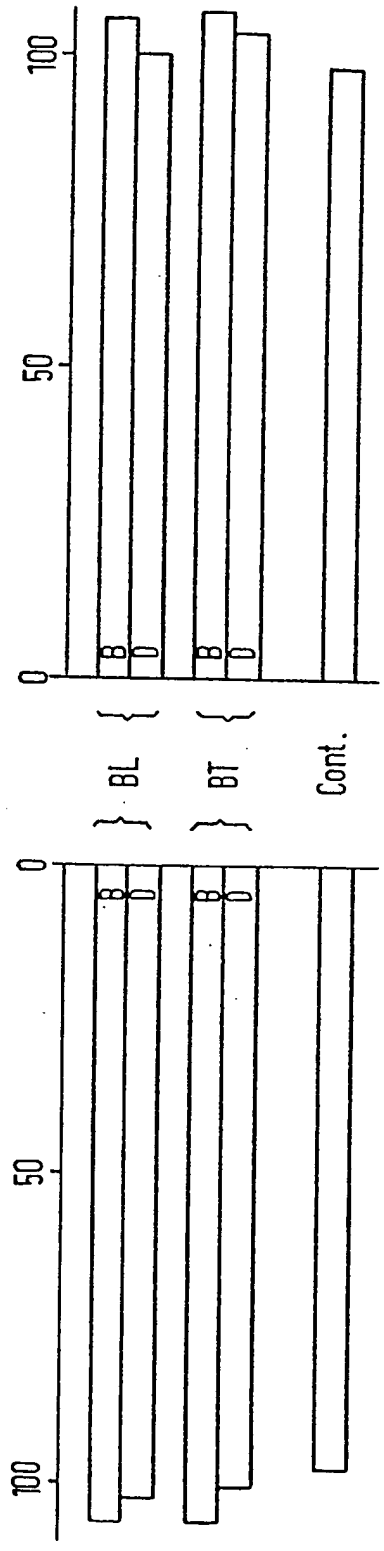


FIG.7

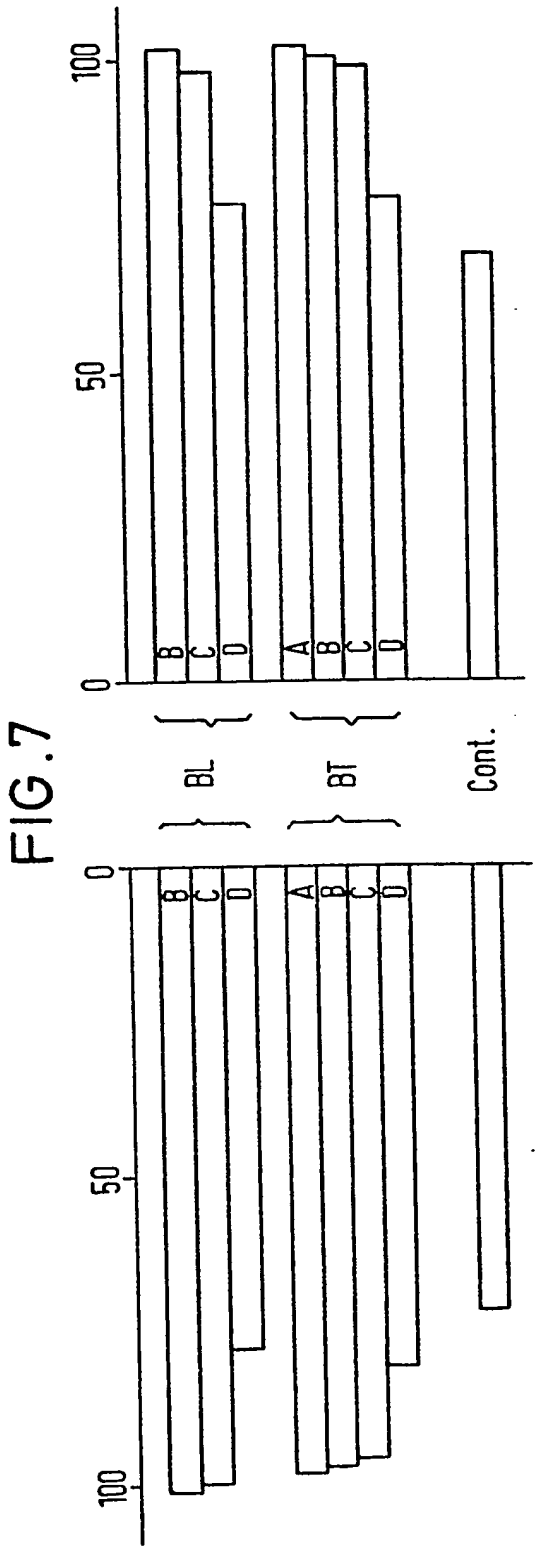


FIG.8

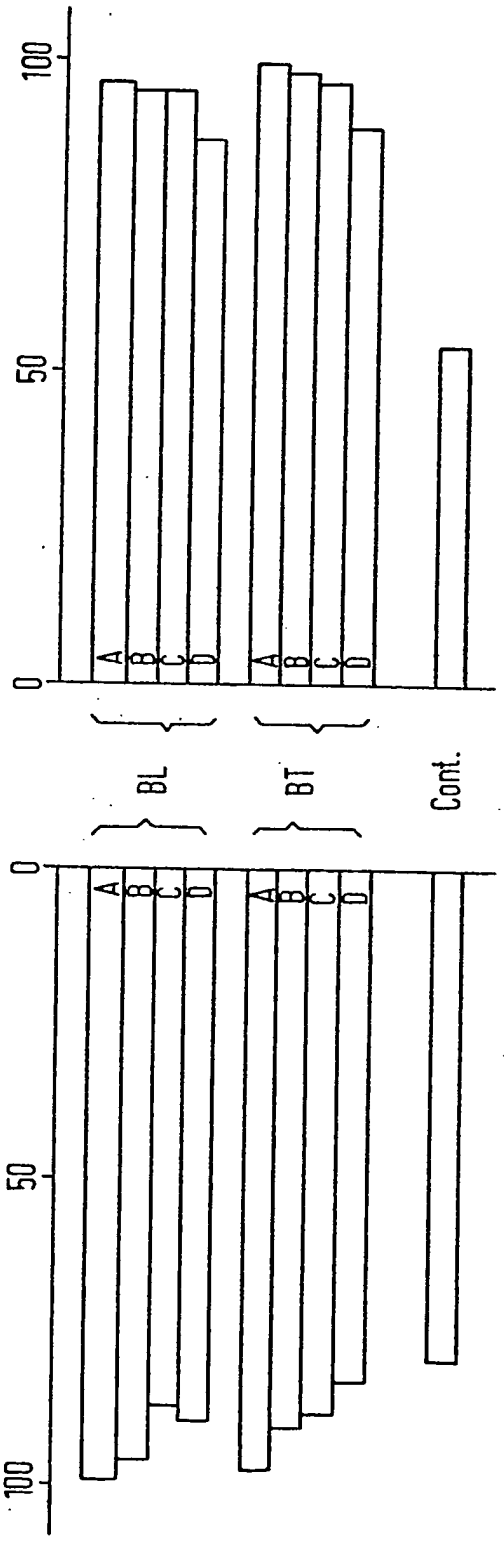


FIG.9

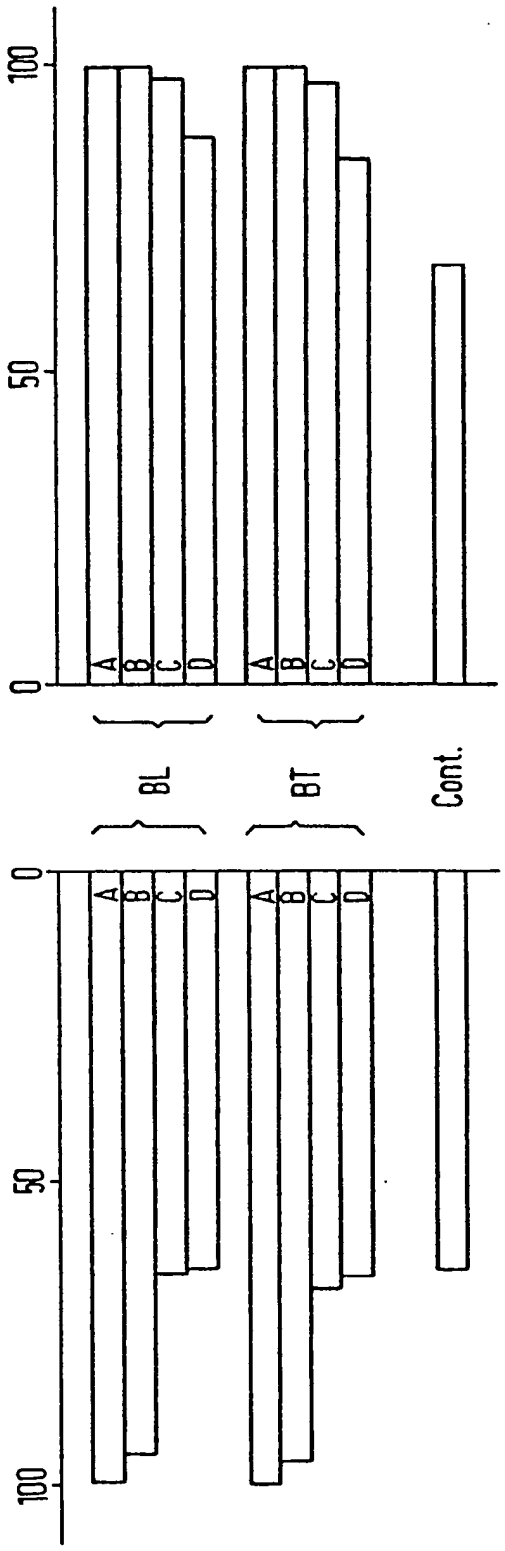
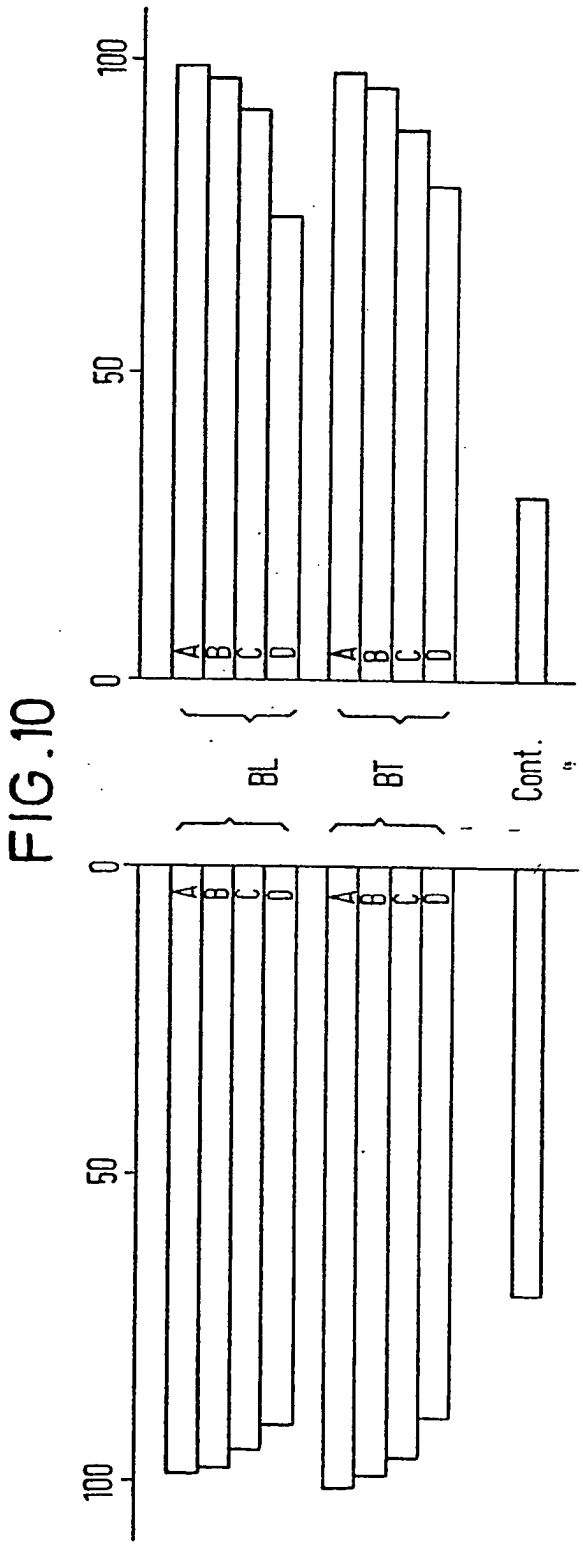


FIG.10



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EUROPEAN SEARCH REPORT

Application number
EP 86 10 4149

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl 4)
X	FR-A-2 156 901 (STANDARD BRANDS INC.) * Page 1, line 1 - page 2, line 36; claims 1-3,9 *	1,3	A 61 K 37/02 A 61 K 47/00
X	GB-A-2 127 689 (SANDOZ) * Page 2, lines 9-36; page 5, line 30 - page 6, line 17; claims 1,2 *	1-4	
X	FR-A-2 325 386 (YAMANOUCHI PHARMACEUTICAL CO. LTD.) * Page 1, lines 1-5; page 8, line 29 - page 9, line 36; page 10, line 38 - page 11, line 1; page 11, line 35 - page 12, line 7; page 20, example 14 *	1-2	
D,A	EP-A-0 095 751 (EISAI CO. LTD.)		

TECHNICAL FIELDS SEARCHED (Int. Cl 4)

A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	18-07-1986	BENZ K.F.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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EUROPEAN SEARCH REPORT

Application Number

EP 91 30 4885

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A,D	WO-A-9001329 (TAKADA K.) * pages 6 - 7 *	1-5	A61K37/02 A61K9/48
A	EP-A-225189 (R.P. SCHERER CORPORATION) * claims 14-19 *	1-5	
A D	EP-A-178665 (CHUGAI) * abstract * & JP-A-61097229	1-5	
A D	EP-A-178576 (CHUGAI) * abstract * & JP-A-61091131	1-5	
A D	GB-A-2177914 (CHUGAI) * abstract * & JP-A-62089627	1-5	
A D D D D	DE-A-3723781 (CHUGAI) * abstract * & JP-A-63146826 & JP-A-63146827 & JP-A-63146828 & JP-A-63152326	1-5	TECHNICAL FIELDS SEARCHED (Int. Cl.5) A61K C07K
A D	EP-A-263490 (CHUGAI) * abstract * & JP-A-63091325	1-5	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	09 AUGUST 1991	AVEDIKIAN P.F.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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L : document cited for other reasons

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